Exhibit C

Bovine Data

Bovine fetal neural stem cells were isolated and cultured essentially as described in Examples 1-5 of the application with the age and developmental time points of the fetus used adjusted accordingly. Fetuses were isolated from freshly killed animals at an abattoir in Poowong, Melbourne, Australia. Fetuses with an approximate crown to rump length of 4-5cm were chosen for the isolation of neural stem cells. The isolation of bovine oocytes and subsequent nuclear transfer procedures are further described below:

Collection of Bovine Oocytes

Bovine ovaries were obtained from a local slaughterhouse, transported at 25-30°C to the laboratory and washed in warmed phosphate buffered saline (PBS, Baxter, Australia). Ovarian antral follicles (2-8mm) were aspirated using an 18-gauge needle and collected into Hepes buffered Tissue Culture Medium 199 (TCM199, Gibco BRL/Life Technologies) with heparin (5000iu/ml, Sigma), 2% Fetal Calf Serum (FCS, Gibco/Life Technologies), and amphotericin B (250µg/ml, Sigma). Cumulus oocyte complexes (COC's) showing an even cytoplasm and surrounded by at least three layers of compact cumulus cells were collected from the follicular fluid. COC's were incubated and matured in groups of 25 in a TCM199 medium supplemented with gentamycin sulfate (10mg/ml), L-glutamine (29mg/ml, Sigma), human Chorionic Gonadotrophin (1500IU/ml, Lyppards, Australia) and 15% FCS at 39°C in 5%CO_2 in air, for 20-24 hours.

Preparation of Oocytes for Nuclear Transfer

In order to remove the surrounding cumulus, matured oocytes at 19-21 hours post maturation (hpm) were vortexed in 80µl maturation media and 20µl hyaluronidase (0.1%, Sigma) for 3 minutes in Eppendorf tubes (Quantum Scientific). The oocytes were washed through handling media (Hepes buffered TCM199 with 5% FCS (199HF)) and those at the metaphase II stage (i.e. with the first polar body extruded) were selected for nuclear transfer (NT).

Nuclear Transfer

Bovine oocytes were enucleated at 20-22hpm in handling media containing cytochalasin B (0.25µl/ml, Sigma) by gentle aspiration of the polar body and metaphase plate in a small amount of cytoplasm using a glass pipette (inner diameter: 10-15µm). The cytoplasts were transferred into TCM199 with 10% FCS and incubated at 39°C in 5% CO_2 in air, until microinjection. After mechanical disruption of the cell membranes in 199HF using the injection pipette, neural stem cells were injected directly into the cytoplasts. The reconstructed embryos were transferred back into TCM199 + 10% FCS until activation.

The reconstructed embryos were activated 30 minutes after microinjection. Embryos were activated in 32µm Ca^{2+} ionophore (Sigma) in TCM199 supplemented with Gentamycin sulfate (10mg/ml) and L-glutamine (29mg/ml) for 10 minutes, followed by a 5-hour incubation in 2mM 6-DMAP (Sigma). Embryos were cultured in modified Synthetic Oviductal Fluid (SOF) culture media supplemented with amino acids (Sigma), 5% FCS, myo-inositol (0.05g/10ml, Sigma) and sodium tri citrate (1mg/1ml, Selby Scientific). Embryos were submerged in a Submarine-Incubation-System (SIS). The 4-well plates were gassed in foil bags (Wests Packaging Services) with 5% O_2, 5% CO_2 and 90% N_2 and immersed in 39°C water for up to six days.

Results

The *in vitro* development results of the bovine nuclear transfer experiments described above are summarised in the following table:

Cell type	N	2-Cell	4-cell	8-cell	Morula	Blastocyst
BFNSC*	250	170	138	115	71	54

*The data were based on sixteen experiments.

BFNSC represent wild-type bovine fetal neural stem cells.

As for the rat and mouse above, the successful development of the reconstructed embryos beyond the 8-10 cell stage (at which point in bovine development becomes dependent on the embryonic genome as opposed to nutrients and proteins derived from the maternal oocyte) to the morula and blastocyst stages demonstrates that reprogramming of the neural stem cell nuclei enabled the expression of the relevant genes required to direct embryonic development. In the bovine, therefore, as for the mouse and rat, neural stem cells represent a viable source of donor nuclei for nuclear transfer experiments.